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Gunnera herteri – developmental morphology of a dwarf from Uruguay and S Brazil (Gunneraceae)

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Abstract. New morphological and developmental observations are presented of *Gunnera herteri* (subgenus *Ostenigunnera*) which is, according to molecular studies, sister to the other species of *Gunnera*. It is an annual dwarf (up to 4 cm long) whereas the other *Gunnera* spp. are perennial and slightly to extremely larger. External stem glands are combined with channels into the stem cortex serving as entrance path for symbiotic *Nostoc* cells. Young stem zones show globular regions of cytoplasm-rich cortex cells, prepared for invasion by *Nostoc*. The leaf axils contain 2–5 inconspicuous colleters (glandular scales) which can be taken as homologous to the more prominent scales of *G. manicata* (subg. *Panke*) and *G. macrophylla* (subg. *Pseudogunnera*). Foliage leaves of *G. herteri* have tooth-like sheath lobes which may be homologous to stipules. Adult plants have extra-axillary inflorescences arising from leaf nodes. The main stem is interpreted as a chain of sympodial units, each one consisting of a leaf and an extra-axillary inflorescence. This "sympodium hypothesis" may be also valid for other species of *Gunnera*. Each globular inflorescence of *G. herteri* contains several female flowers and 2–7 stamens at the top, perhaps equalling a single male flower. There are neither bracts nor bracteoles. The ovary is inferior, bicarpellary and unilocular. Its single hanging ovule develops into a dry and endosperm-rich seed.

Key words: *Gunnera, Ostenigunnera, Panke, Nostoc,* axillary glands, basal eudicots, congenital fusion, development, sympodial growth, unisexual flowers.

Developmental morphology of *Gunnera herteri*.

Gunnera is a genus of flowering plants that includes 30–40 species with a mainly southern distribution. Schindler (1905) divided *Gunnera* into five subgenera based on the size of the plants, their means of propagation and their geographical distribution. Mattfeld (1933) created the new subgenus *Ostenigunnera* to include a new species of *Gunnera*, discovered in a small oasis among the sand dunes of the Bay of Rocha (Uruguay). This species was named *G. herteri* to honour W. Herter, who together with C. Osten found and collected the plant (see Osten 1932). Morphological and anatomical studies on the shoot and the vascular systems, respectively, together with the presence of symbiotic cyanobacteria of the genus *Nostoc*, showed that the tiny plant really belonged to the genus *Gunnera* (Mattfeld 1933).

Gunnera herteri is a rare plant with a very restricted distribution. Except for the district

of Rocha in Uruguay, the plant occurs in similar environments in the adjacent districts of Rio Grande and Santa Catarina (Brazil) where it is called "Urtiguinha das Dunas". These very few localities for *G. herteri* are endangered by the constructions of tourist resorts on the beaches and by pine plantations planted to stop the movement of the sand. Due to its narrow distribution, *Gunnera herteri* has been collected very sparsely during the past hundred-and-ten years. The first collection of *G. herteri* was made by the Swedish botanists Lindman and Malme, twice in Santa Catarina, as early as in 1892 and in 1901, respectively, but their herbarium specimens remained unnamed for more than 30 years at the Swedish Museum of Natural History. *Gunnera herteri* has to the best of our knowledge only been collected in Brazil twice since that time. In Uruguay the plant has also been collected twice since its first discovery; once by E. Paz in 1989 and again by L. Wanntorp (the second author of this paper) in 1997.

Thanks to the extensive research of the past few years on the systematics of *Gunnera*, new data on *G. herteri* have come to light. The cultivation of *G. herteri* proved that the plant is not a perennial as stated by older literature (Osten 1932, Mattfeld 1933). In fact, it is the single annual species within the genus. In phylogenetic studies on the genus, based on both molecular and morphological data (Wanntorp and Wanntorp 2003; Wanntorp et al. 2001, 2002, 2003), *G. herteri* was found as sister to the remaining species of the genus. This phylogenetic position is useful for clarifying the historical biogeography of this species. South America is often considered as a composite area in biogeographic studies (Wanntorp and Wanntorp 2003). The area where *G. herteri* occurs today was, together with West Africa, part of an extensive area united along the Guinea fracture zone until about 105 mya (Albian, Cretaceous). In light of this, *Gunnera herteri* could be interpreted as a relict taxon from that area (Wanntorp and Wanntorp 2003).

The morphology of mature plants of *G. herteri* was studied by Mattfeld (1933). Various questions about reproductive structures and the developmental morphology of the plant could not be elucidated due to the scarce material available. Recently, the vegetative anatomy of *G. herteri* was examined for the first time by Wilkinson (2000) in a study mainly based on light microscopy of herbarium material. Except for these studies, no comprehensive morphological study based on SEM has so far been presented and there is at present no study on the flower morphology of *G. herteri*.

The principal aim of this study is to present the developmental morphology of *G. herteri* in detail by using SEM and microtome/light microscope techniques on fresh material. A brief comparison between some morphological and anatomical characters of *G. herteri* and some other species of *Gunnera* is here also reported.

Material and methods

- *Gunnera herteri* Osten (subgenus *Ostenigunnera*): fruits and alcohol-fixed material collected 1997 in Uruguay by L. & H-E. Wanntorp 555 (S); other fresh material was cultivated in the Botanical Garden of Stockholm.
- *Gunnera manicata* Linden (subgenus *Panke*): cultivated in the Botanical Garden of Zurich University (since 1949, source unknown).

The plant specimens used were fixed and preserved in 70% ethanol. Preserved material stored in 70% ethanol was used for light and scanning electron microscopy (SEM). For microtome sections, specimens were embedded in Kulzer's Technovit (2-hydroethyl methacrylate), as described in Igersheim and Cichocki (1996), and sectioned with a HM 355 rotary microtome and conventional microtome knife type C and D. The mostly 7 μm thick sections were stained with ruthenium red and toluidine blue. The permanent slides of the microtome sections are deposited at the Institute of Systematic Botany of the University of Zurich (Z).

Results

Habit. *Gunnera herteri* is a tiny annual, forming dense mats on seepage ground between the coastal dunes of Uruguay and southern Brazil. It has branched stems 2–4 cm long (diameter 0.5–1 mm) with spirally arranged foliage leaves (Figs. 8, 35, 64). There are no stolons. Vegetative lateral shoots arise from the leaf axils and repeat the upright growth of the main stem. In addition, there are 1(–2) cm long stalked tiny inflorescences (one per leaf). They are inserted extra-axillary, on the right or left side of the insertion area of a foliage leaf (Fig. 40). Most roots are secondary (''adventitious'') arising endogenously from the stem whereas the primary root (radicle) seems to wither soon (Figs. 3, 4).

Anatomy of stems and roots. Transverse stem sections show a central stele, consisting of a hollow vascular cylinder with lacunae for the entering leaf traces (Figs. 1, 13). Occasionally there is additional vascular tissue in the parenchymatous pith (Figs. 2, 16). The surrounding parenchymatous stem cortex contains cavities which are filled with endogenous roots and *Nostoc* colonies. Many endogenous roots protrude from the surface of the stem base, from nodes as well as internodes (Figs. 3, 4) while there are no adventitious roots higher up. Each root is provided with a prominent cap (Figs. 5, 6). The root cortex shows narrow peripheral cells and wider cells towards the center. The central cylinder is triarch and surrounded by a clear endodermis (Fig. 7; see Wilkinson 2000, her figs. 51, 52).

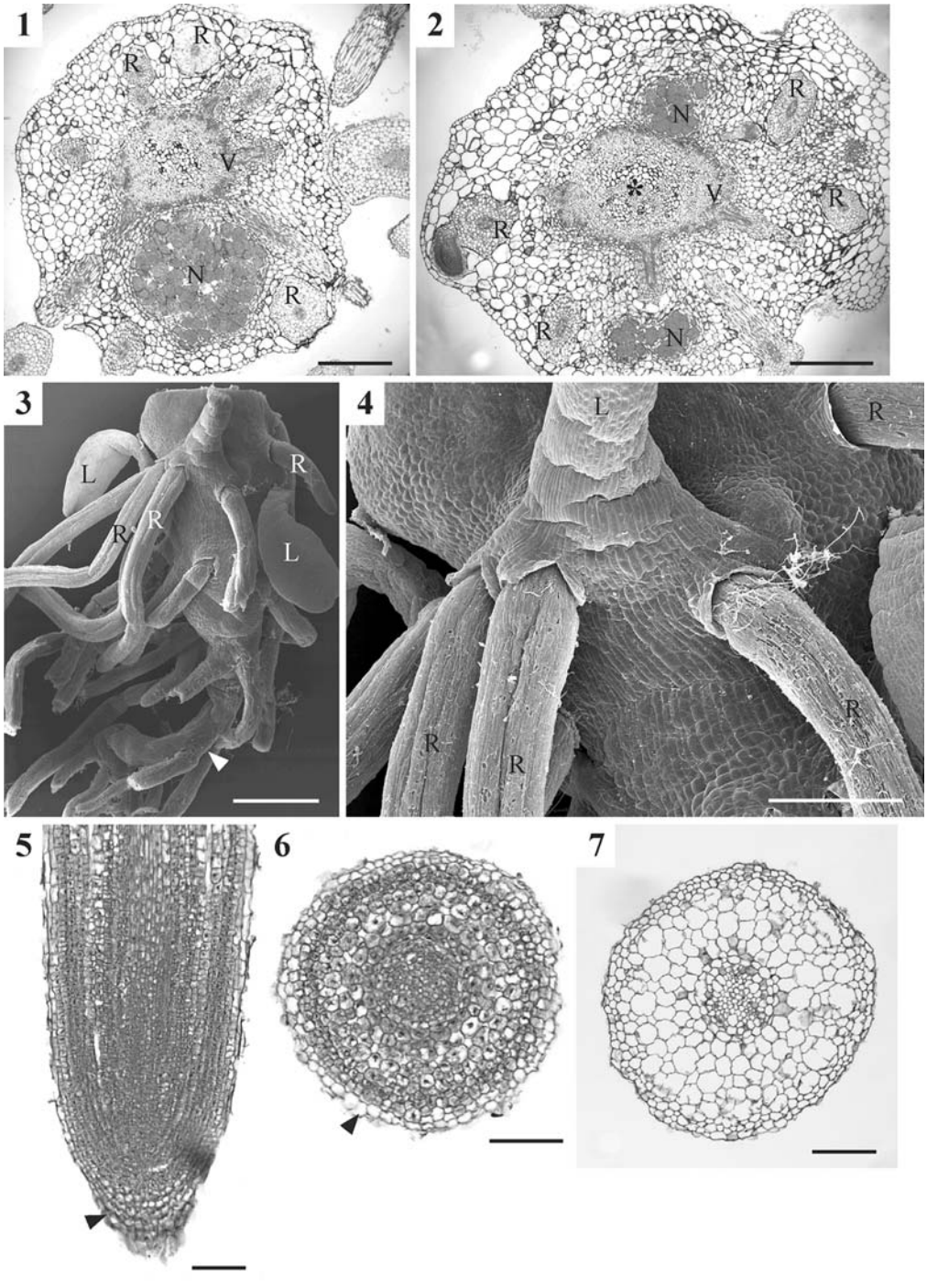
Anatomy of stem glands and *Nostoc* infection channel. Stem glands are observable next to the leaf insertion areas (Figs. 8 – 12). The mucilage-producing glands which serve as entrance path for *Nostoc* cells (Uheda and Silvester 2001) are funnel-shaped with a distinct rim and a somewhat papillate surface (Fig. 10). Transverse sections of young stem portions show a channel that leads from the external glandular rim into the inner stem cortex (Figs. 13, 14). This channel is lined with cytoplasma-rich cells. Young stem zones have globular regions of cytoplasma-rich cortex cells which are prepared for being invaded by *Nostoc* (Fig. 15). After infection *Nostoc* proliferates within the cortex cells (Fig. 16). There are intercellular cavities in the surrounding stem cortex.

Morphology and development of foliage leaves. Fully grown foliage leaves consist of a stalk 6–7 mm long and a cordate to kidney-shaped blade which is crenate or slightly lobed (up to 4 mm long, 7 mm broad, Fig. 64). Each blade lobe (or crenation) is topped by a marginal hydathode (Figs. 24, 25). The palmate blade venation divides up into fine reticulate vascular bundles (Fig. 28). Anomocytic stomates are found on both the upper and lower blade surface (Figs. 26, 27). The blade mesophyll consists of a layer of moderately elongated palisade cells and 1–3 layers of spongy cells (Fig. 29). The young blade in the bud stage shows involute vernation, i.e. the lateral blade portions are rolled towards the upper (ventral) side (Figs. 9, 23). Three petiole bundles enter the blade (Figs. 35, 38). They are fused into one leaf trace near the petiole insertion (Fig. 39). Each petiole base is usually broadened into a sheath with two attached lobes resembling lateral stipules (Figs. 12, 17, 20). Sheath lobes of young leaves next to the terminal bud border the early present *Nostoc* glands (Figs. 8–11). Primary leaves (including seedling leaves, cotyledons) are entire, lanceolate and lacking sheath lobes (Figs. 3, 4).

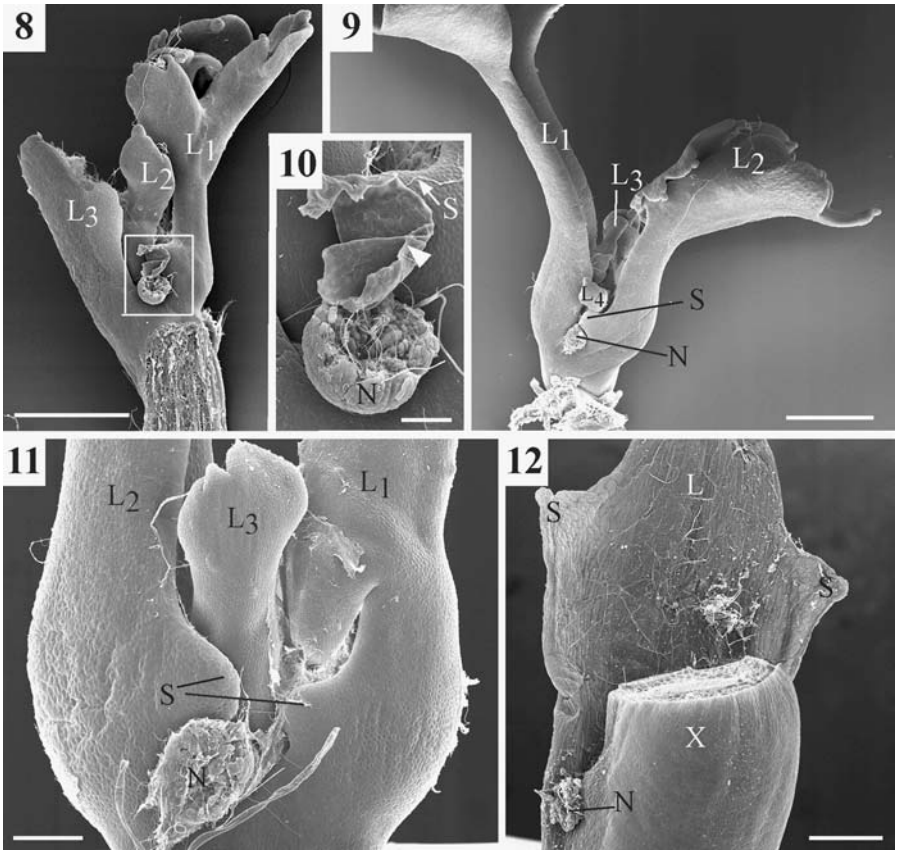
Vegetative axillary buds and axillary colleters. Young foliage leaf axils are occupied by a vegetative lateral bud consisting of a shoot apex and the first two leaf primordia (prophylls) which are inserted in an oblique position approaching the median plane (Figs. 17 – 19). These axillary buds, however, are not the only axillary outgrowths. On each side of the axillary bud there is at least one colleter (glandular scale) adjacent to the vegetative lateral bud but not being part of it (C in Figs. 18, 19). These axillary colleters develop before the vegetative bud. Thus, certain young leaf axils can have two or three colleters but no or

only a rudimentary vegetative bud in between (Figs. 20, 21, 22). Occasionally there may be up to four or five colleters per leaf axil (Fig. 38, see also Mattfeld 1933).

Architecture and development of inflorescences, flowers and fruits. A plant may produce 3–10 stalked inflorescences, each one consisting of 8–30 flowers in a compact cluster (Figs. 41, 42). Only 2–7 stamens are found at the tip of each inflorescence, arising from a common stalk (Figs. 43, 44). According to Mattfeld (1933) the male flowers are

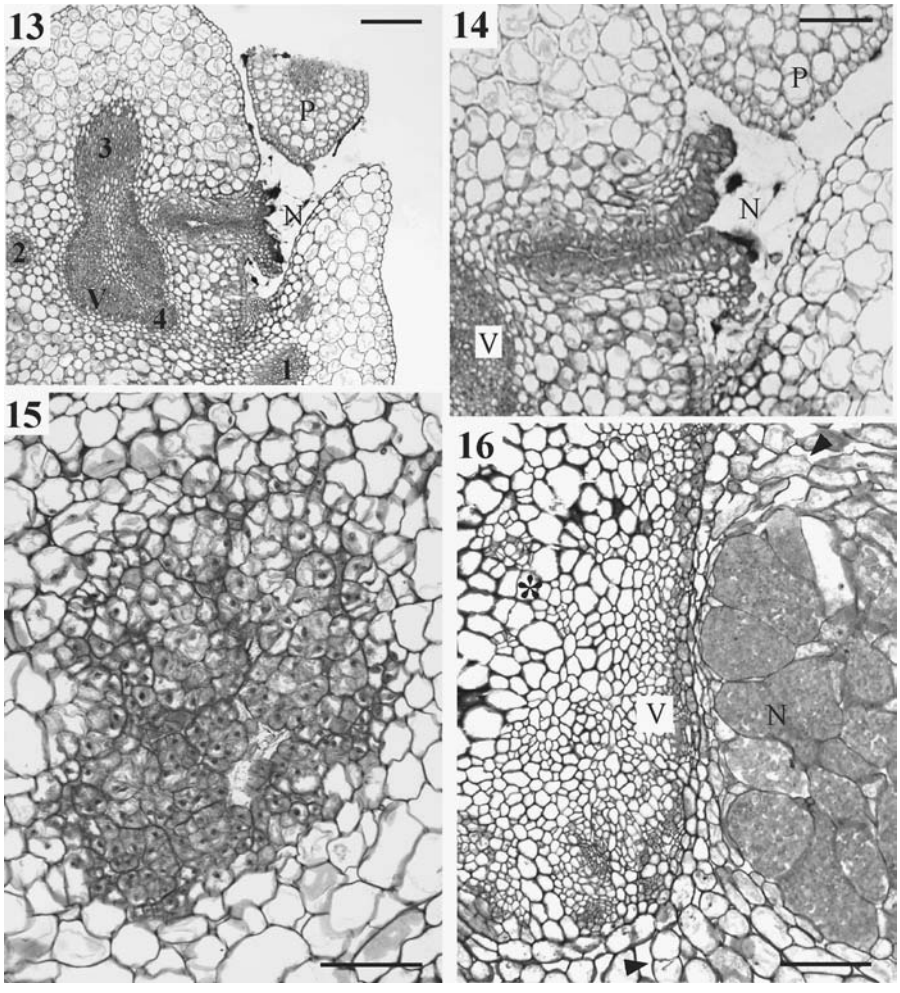


highly reduced, with each single stamen equalling a male flower. Another view allows to accept all stamens as part of a single terminal male flower (see Discussion). All other flowers in the inflorescence, i.e. up to more than 20, are female ones (Figs. 41, 42). Very small inflorescences may consist of only two female flowers and no male flower at all



Figs. 8–12. *Gunnera herteri* (cult. BG Stockholm). Vegetative shoot tips with leaf bases and *Nostoc* glands. **8, 9.** Two shoot tips with three and four observable leaves (*L1 – L4*), each provided with petiole and blade. Note presence of basal sheath lobe (*S*) above *Nostoc* stem gland (*N*). Scale bars = 900 μm. **10.** Close-up of fig. 8. Funnel-shaped stem gland (*N*) with rim serving as entrance path for *Nostoc* cells. Arrowhead points to mucilage between stem gland (*N*) and sheath-lobe (*S*) of leaf 1. Scale bar = 100 μm. **11.** Close-up of shoot tip shown in fig. 9, seen from backside. Leaves 1 and 2 provided with sheath lobe just above *Nostoc* stem gland (*N*) which is partly covered by mucilage. Scale bar = 200 μm. **12.** Stem portion (*X*) cut above insertion level of leaf (*L*) which is provided with two sheath lobes (*S*). Note presence of *Nostoc* stem gland (*N*). Scale bar = 400 μm

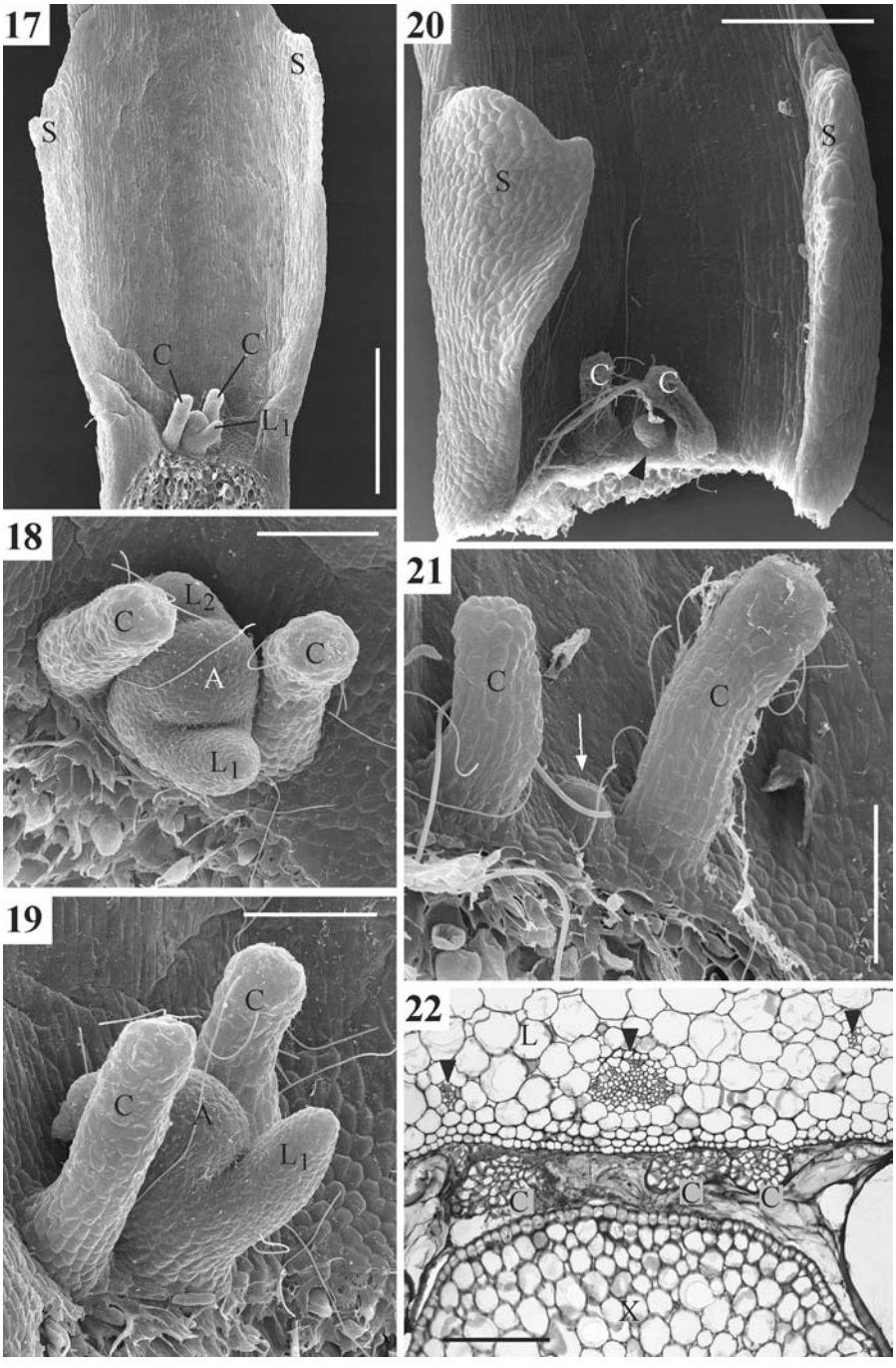
Figs. 1–7. *Gunnera herteri* (cult. BG Stockholm). Stems and roots. **1, 2.** Two cross-sections of lower stem portion of 2 cm high plant (including leaves). Central stele (*V*) with additional vascular tissue (asterisk) in parenchymatous pith. Surrounding parenchymatous cortex with cavities filled with endogenous roots (*R*) and *Nostoc* colonies (*N*). Scale bars = 700 μm. **3, 4.** Overview and close-up of lower stem portion with leaves (*L*) and endogenous roots (*R*) arising from stem cortex and leaf base. Arrowhead points to primary root. Scale bars = 1 mm and 500 μm, respectively. **5**. Longitudinal section of root tip. Arrowhead points to root cap (calyptra). Scale bar = 100 μm. **6, 7.** Cross-sections of meristematic root tip (surrounded by cap, see arrowhead) and of a differentiated root zone, respectively, showing triarch structure of central cylinder. Scale bars = 100 μm



Figs. 13–16. *Gunnera herteri* (cult. BG Stockholm). *Nostoc* infection path and colonies. **13, 14.** Two cross-sections (overview and close-up) cut at slightly different levels of young stem, showing funnel-shaped gland (*N*) and channel into stem cortex prior to infection by *Nostoc* (same shoot tip is shown in figs. 38, 39). Vascular traces of leaves 1–4 entering vascular stele (*V*) of stem in a spiral pattern. *P* = stalk (peduncle) of extra-axillary inflorescence. Note presence of cytoplasma-rich cells along infection channel. Scale bars = 200 μm. **15.** Young stem cortex region showing tissue nodule with cytoplasma-rich cells at inner end of infection channel, not yet infected by *Nostoc*. Scale bar = 200 μm. **16.** Older stem portion with cortex cells filled with *Nostoc* cells next to vascular stele (*V*). Note tiny vascular strands (asterisk) in parenchymatous pith inside vascular stele (*V*). Arrowheads point to intercellular cavities in stem cortex. Scale bar = 100 μm

(Fig. 40). The pollen grains are tricolpate, the exine consists of sinuous muri forming an imperfect reticulum (Figs. 57–59; see also Wanntorp et al. 2004).

Young inflorescence buds are provided with lateral bumps (subunit primordia) which arise acropetally in a spiral pattern (Figs. 33, 34). The female flowers arise from lateral inflorescence subunits which are triads (Figs. 41, 42) or secondary spikes, as observable in young stages with acropetally initiated floral primordia (Fig. 45). The inflorescences tend to be protandrous (as already mentioned by Osten 1932), with the apical stamens (male flower) being differentiated and shedding the pollen early while the lateral female flowers are still small and immature (Figs. 32, 36, 44–46). The whole inflorescence lacks any bracts

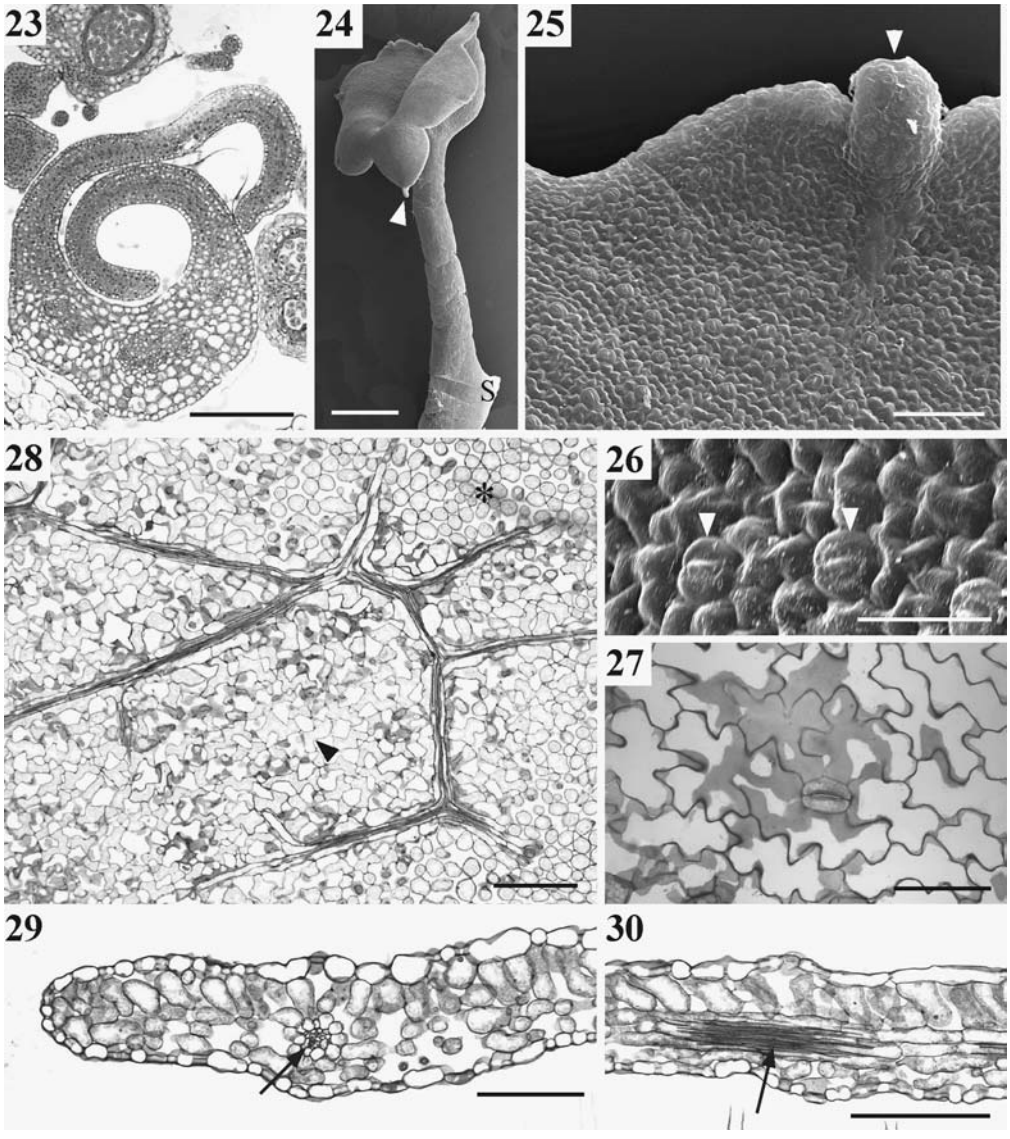


Figs. 17–22. *Gunnera herteri* (cult. BG Stockholm). Adaxial views of foliage leaf bases with axillary buds and adjacent scales (glandular colleters). **17, 18, 19.** Overview and two close-ups of leaf base (clearly below sheath-lobes *S*) with axillary bud consisting of apical meristem (*A*) and two leaf primordia (*L1, L2*). There is one glandular scale = colleter (*C*) on each side of the axillary bud. Scale bars = 500 μm (Fig. 17), 90 μm (Figs. 18, 19). **20.** Another leaf base with two sheath lobes (*S*). The rudimentary axillary bud (arrowhead) is partly hidden by two scales (*C*) and mucilage. Scale bar = 200 μm. **21.** Another leaf base. Except for a small bump (arrow) no axillary bud is observable between the two scales (*C*). Scale bar = 100 μm. **22.** Cross-section of stem (*X*) slightly above insertion of leaf (*L*). The axillary cleft is filled with mucilage produced by three scales (*C*). Arrowheads point to three vascular bundles of leaf base. Scale bar = 200 μm

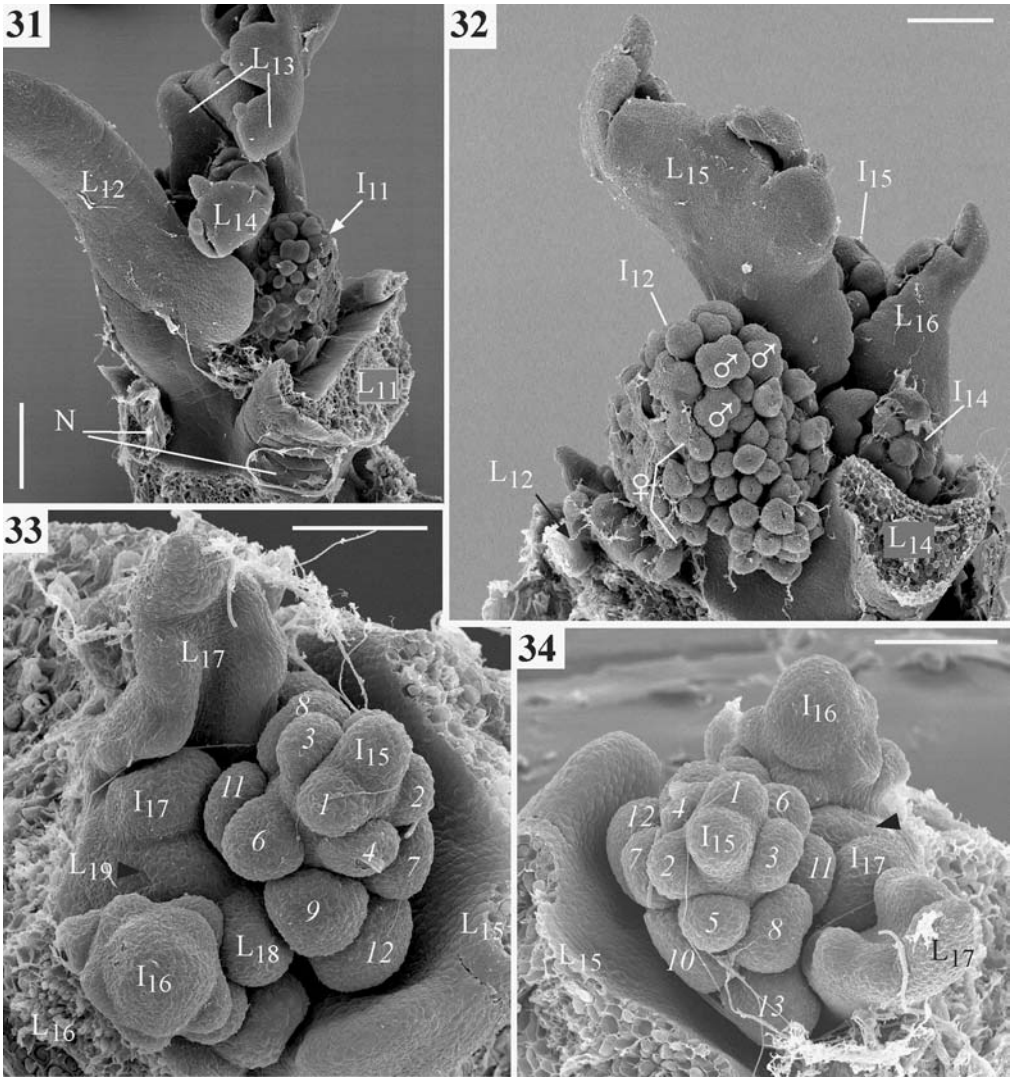
(subtending leaves) and bracteoles (prophylls). There are vascular bundles which are derived from the stele of the inflorescence axis and which divide up again in order to provide each flower with a single strand (Fig. 48).

Each female flower is reduced to an inferior ovary on a short stalk, topped by two papillate stigma lobes[i] and two tooth-like tepals arranged in a plane rectangular to the plane of the stigma lobes (Figs. 49–52). There are two

[i]The stigma lobes in *Gunnera* are called styles by Endress and Igersheim (1999, p. 353): They wrote: "2 free styles, the stigma extends along the entire surface of the free styles".



Figs. 23–30. *Gunnera herteri* (cult. BG Stockholm). Developmental and anatomical aspects of foliage leaves. **23.** Young foliage leaf with involute blade vernation. Scale bar = 200 μm. **24.** Oblique abaxial view of young seedling leaf with palmately lobed blade on elongate petiole. Arrowhead points to marginal tooth with hydathode. Note presence of sheath-lobe (*S*) at leaf base. Scale bar = 1 mm. **25.** Overview of upper ("adaxial") epidermis near blade margin. Note marginal hydathode (arrowhead). Scale bar = 100 μm. **26.** Close-up of upper epidermis with stomates (arrowheads). Scale bar = 40 μm. **27.** Another portion of upper blade epidermis, seen as surface view. Note presence of stomate surrounded by epidermal cells with wavy outlines (anomocytic type). Scale bar = 100 μm. **28.** Paradermal section of blade portion, showing network of tiny vascular bundles, palisade parenchyma (asterisk) and spongy parenchyma (arrowhead). Scale bar = 200 μm. **29, 30.** Marginal and more proximal cross-section of leaf-blade, respectively. The chlorenchyma consists of single-layered palisade parenchyma and up to three layers of spongy parenchyma. Arrows point to vascular bundles in transverse and longitudinal sections. Scale bars = 200 μm

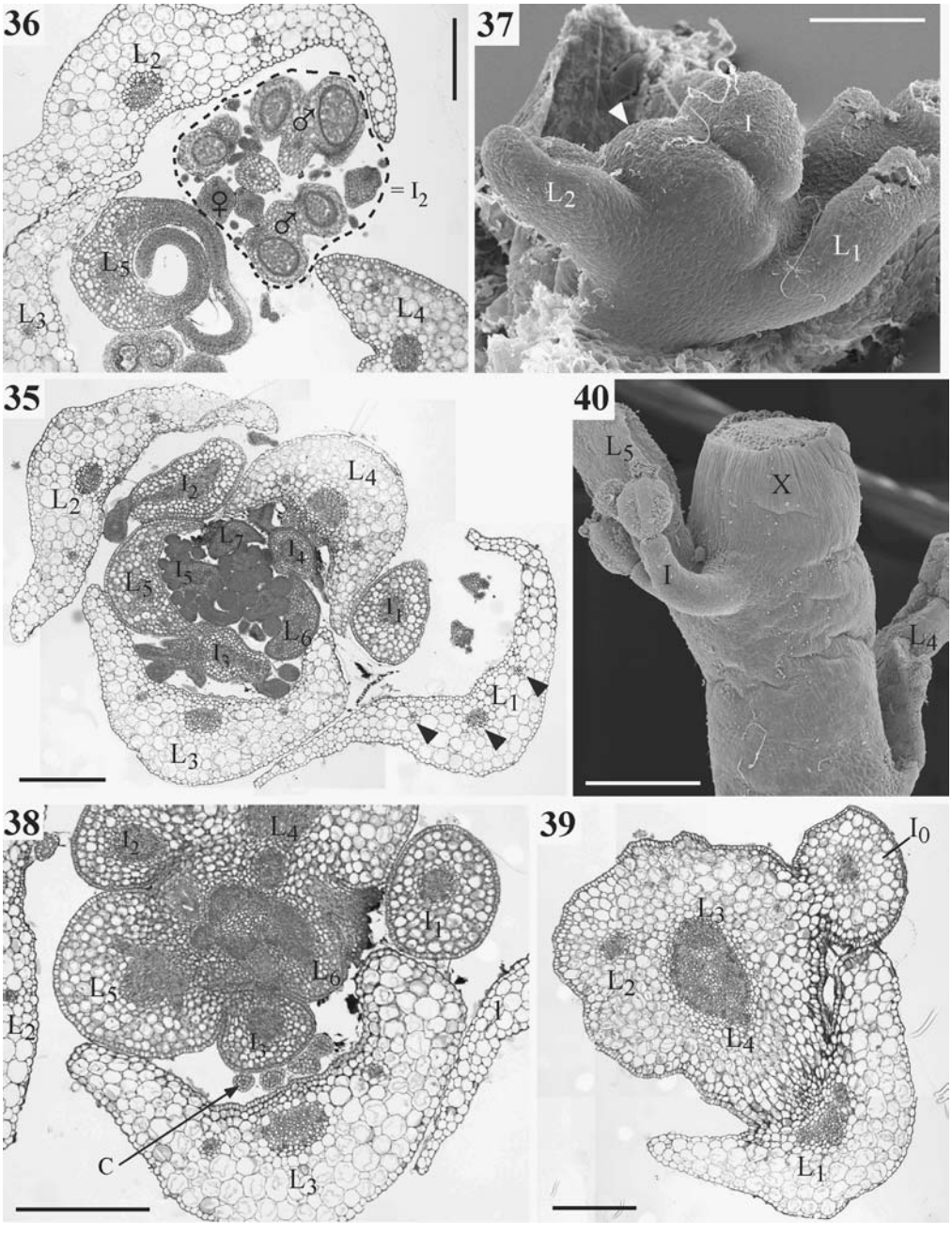


Figs. 31–34. *Gunnera herteri* (cult. BG Stockholm). Young inflorescences in shoot tip region. **31.** Shoot tip with spirally arranged young leaves (*L11–L14*). Young inflorescence (*I11*) seemingly in axil of leaf *11* (removed). Note presence of *Nostoc* glands (*N*). Scale bar = 400 μm. **32.** Same shoot tip as above, after removal of leaves *12–14*. Inflorescence *I12* (seemingly in axil of leaf *12*) with few male flower buds = stamens (♂) above and many female flower buds (♀) below. Younger inflorescence *I14* seemingly in axil of leaf *14*. Scale bar = 200 μm. **33, 34.** Same shoot tip as above, after removal of leaves *L15* and *L16* in order to better observe their "nearly axillary" inflorescences (*I15, I16*), seen from two sides. Inflorescence buds *I15* and *I16* are totally or partly divided up into reproductive bumps lacking subtending bracts. Numerals *1–13 (in italics)* indicate spiral initiation pattern of subunits of inflorescence (*I15*). Arrowheads point to position of inconspicuous shoot apical meristem. Note somewhat extra-axillary position of inflorescence primordium *I17* bordering shoot apical meristem in front of leaf *17*. Scale bars = 100 μm

vascular bundles in the ovary wall ending up in the two tepals (Fig. 53). Early developmental stages of female flowers are provided with a two-cornered rim, indicating the two tepal primordia while the initial inferior ovary is observable as slit in the center (Figs. 47, 55). This area gives rise to the pollen tube transmitting tissue providing the single and pendent ovule (Figs. 51, 56). The ovary is bicarpellary and unilocular, with a single hanging ovule.

Young fruits (i.e. postanthetic female flowers) start to differentiate their ovary wall into a small-celled inner layer (forming later the stony endocarp) and a large-celled outer layer (forming later the slightly fleshy mesocarp of the drupe-like fruit, Figs. 53, 54). The young drupe contains abundant endosperm (as also described by Webb and Simpson (2001) for New Zealand *Gunnera* spp.), with the young embryo provided with one or two large suspensor cells (Figs. 53, 54).

Position of the inflorescences as compared to the leaf insertion sites. Mature stem portions show the inflorescences as extra-axillary lateral outgrowths with a naked stalk (peduncle), not subtended by a foliage leaf nearby. There is always an adjacent leaf which is inserted on the same level (same stem node) as



the inflorescence; leaf and adjacent inflorescence occupy nodal sectors which deviate 90° or less (Figs. 38–40, 62, 64). In young stages, however, each inflorescence seems to be inserted in or near the axil of a "subtending leaf" (Figs. 31–34). While the stem is elongating, it becomes obvious that the inflorescence stalk (I3) is inserted clearly above its "subtending leaf" (Fig. 38). Each inflorescence seems to become extra-axillary by shifting along the elongating stem. Shifting (also called congenital fusion) and torsion of a monopodial stem were accepted by Mattfeld (1933). His explanation will be questioned in the Discussion under the heading "sympodial hypothesis of stem growth". Mattfeld's interpretation does not really fit with the exact arrangement of leaf and inflorescence primordia observable in the shoot tip, next to the shoot apical meristems (Figs. 33, 34). For example, the primordial inflorescence I17 does not exactly occupy an axillary position in front of leaf L17. The inflorescence bud I17 already occupies a slightly oblique (i.e. somewhat extra-axillary) position bordering the shoot apical meristem (see arrowhead in Figs. 33, 34). Some inflorescences soon become strongly extra-axillary by an asymmetric growth of the upper inflorescence parts (Figs. 35, 36).

In axillary buds the switch from the vegetative to the reproductive growth phase may happen quite early, i.e. already after the formation of the first two leaves (prophylls). For example in Fig. 37, showing an axillary bud with two leaves, the first inflorescence primordium is already initiated. According to Mattfeld's (1933) interpretation this primordial inflorescence (I) is a bud in the axil of leaf L1. However, looking more carefully at Fig. 37 we realize that the primordial inflorescence is initiated clearly above leaf axil 1 and in a slightly extra-axillary position, i.e. not exactly in the median plane of leaf 1. Another more suitable interpretation (see Discussion under heading "sympodial hypothesis of stem growth") allows the acceptance of the primordial bump I (Fig. 37) as a terminal inflorescence of the first shoot order consisting of two leaves (L1 and L2) and an inflorescence. Then, an axillary meristem is formed in the axil of leaf 2 giving rise to the next (second) sympodial unit.

Discussion

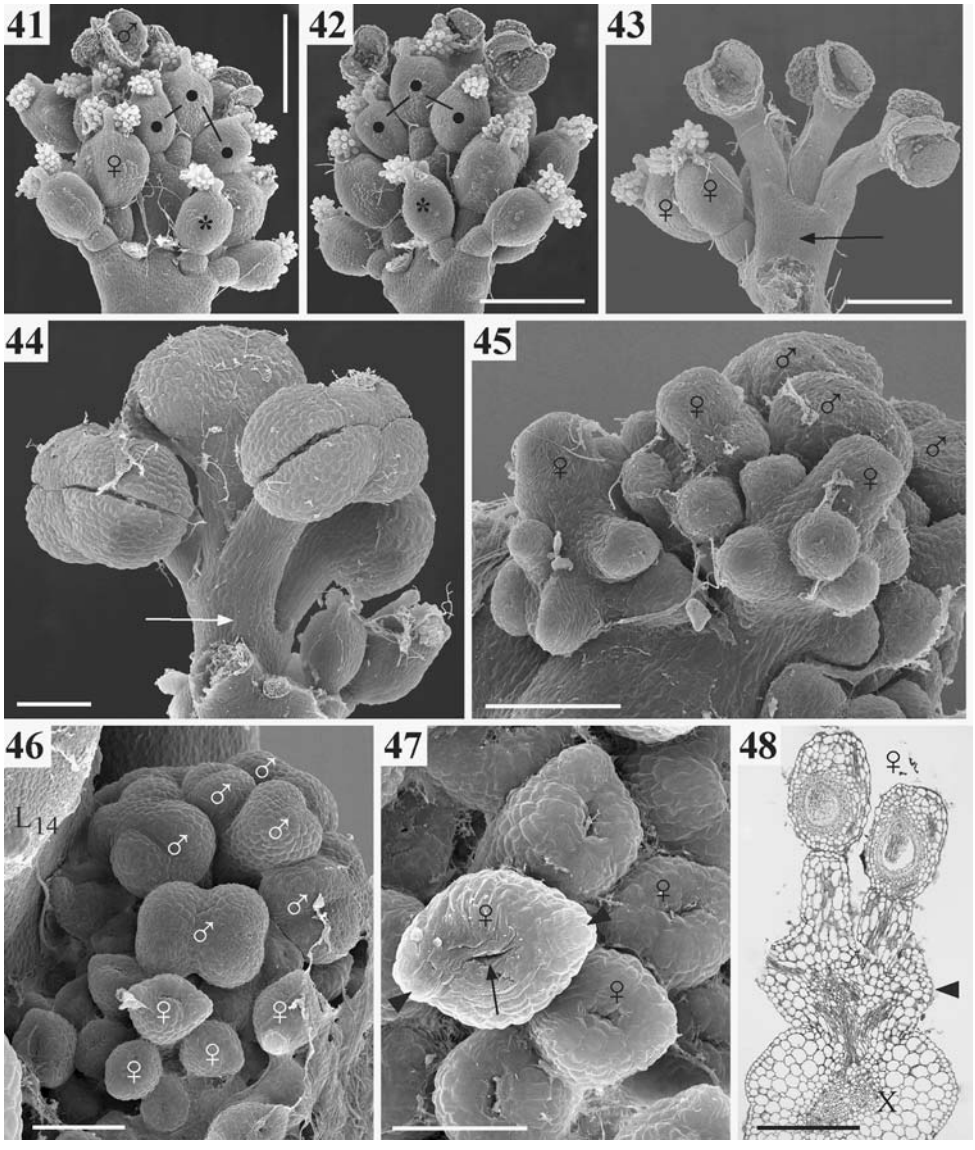
Molecular data (e.g. Savolainen et al. 2000, Soltis et al. 2003) revealed that the Gunnerales (including *Gunnera* and *Myrothamnus*) are sister to all core eudicots. According to the molecular phylogeny of *Gunnera* (Wanntorp and Wanntorp 2003; Wanntorp et al. 2001, 2002, 2003) the annual dwarf species *Gunnera herteri* is sister to all other species of *Gunnera*. As a preliminary evolutionary hypothesis, we may accept the first *Gunnera* members (similar to *G. herteri*) as small to mid-sized herbs whereas the giant rosette herbs (subgenus

Figs. 35–40. *Gunnera herteri* (cult. BG Stockholm). Cross-sections and SEM graph showing inflorescence positions (*I*) as compared to spiral leaf arrangement (*L1 – L7*). **35.** Overview of whole shoot tip with spirally arranged foliage leaves (*L1–L7*) and "nearly axillary" inflorescence buds (*I1–I5*). Each leaf base is provided with three vascular bundles (arrowheads). Note slightly oblique ("nearly axillary") positions of inflorescence stalks (*I1*, *I2*) in axils of leaves *L1* and *L2*, respectively. Scale bar = 450 μm. **36.** Close-up of cross-section through shoot tip clearly above fig. 35, showing oblique ("nearly axillary") position of inflorescence *I2*, surrounded by broken line. The inflorescence is cut in distal portion, showing stamens (♂) and female flower buds (♀). Scale bar = 250 μm. 37. Vegetative axillary bud with first leaves (prophylls) *L1* and *L2*, lateral shoot meristem in axil of leaf *L2* (arrowhead); precursive formation of inflorescence primordium *I* in slightly oblique ("nearly axillary") position in front of leaf *1*. Scale bar = 100 μm. **38, 39.** Cross-sections through shoot tip clearly below fig. 35, showing extra-axillary position of inflorescences (*I*). Insertion of inflorescence stalk *I3* is clearly above insertion level of foliage leaf *L3*, and insertion of inflorescence *I0* on same level as leaf *L1*. Note presence of four scales = colleters (*C*) in axil of leaf 3. Scale bars = 400 μm. **40.** Seedling axis (*X*) with bases of leaves *4* and *5* and small extra-axillary inflorescence (*I*, consisting of two female flowers). Scale bar = *1* mm

Panke) with leaves up to 2 m high and inflorescences up to 1 m long are derived. *Gunnera herteri*, however, may have undergone various morphological reductions or losses, possibly due to miniaturization. These reductions or losses are then best viewed as autapomorphies as compared to mid-sized *Gunnera* members. There are extremely large morphological differences between *Gunnera* and its sister genus *Myrothamnus* which is perennial as most *Gunnera* spp. (see Endress 1989, Endress and Igersheim 1999, Wilkinson 2000, Soltis et al. 2003).

In discussing our new data we will emphasize those points that shed light on the interpretations already presented by Mattfeld (1933). He put *G. herteri* in the subgenus *Ostenigunnera*, clearly distinguishable from the other five subgenera already known before. This decision was based on the following characters (see also Table 1):

(1) *Gunnera herteri* lacks stolons, especially as compared with the members of the subgenus *Milligania* which form small but perennial herbs.



(2) The vascular stem tissue in *G. herteri* is very simple, mainly consisting of a vascular cylinder or 3–4 separate bundles arranged in a ring. Mattfeld (1933) pointed to similarities with the stolons of *G. dentata* (subgenus *Milligania*) which have a vascular tissue as simple as the stem stele of *G. herteri* (see also Schnegg 1902, Schindler 1905).
(3) There are elongated upright stems with extra-axillary lateral inflorescences (as discussed below in paragraph I).
(4) There is a group of 2–7 stamens at the top of each inflorescence while all flowers below are female. According to Mattfeld (1933) each male flower of *G. herteri* consists of a single stamen without any rudiment of perianth and gynoecium.

Our morphological analysis of mature parts of *G. herteri* has revealed some characters not described by Mattfeld (1933):

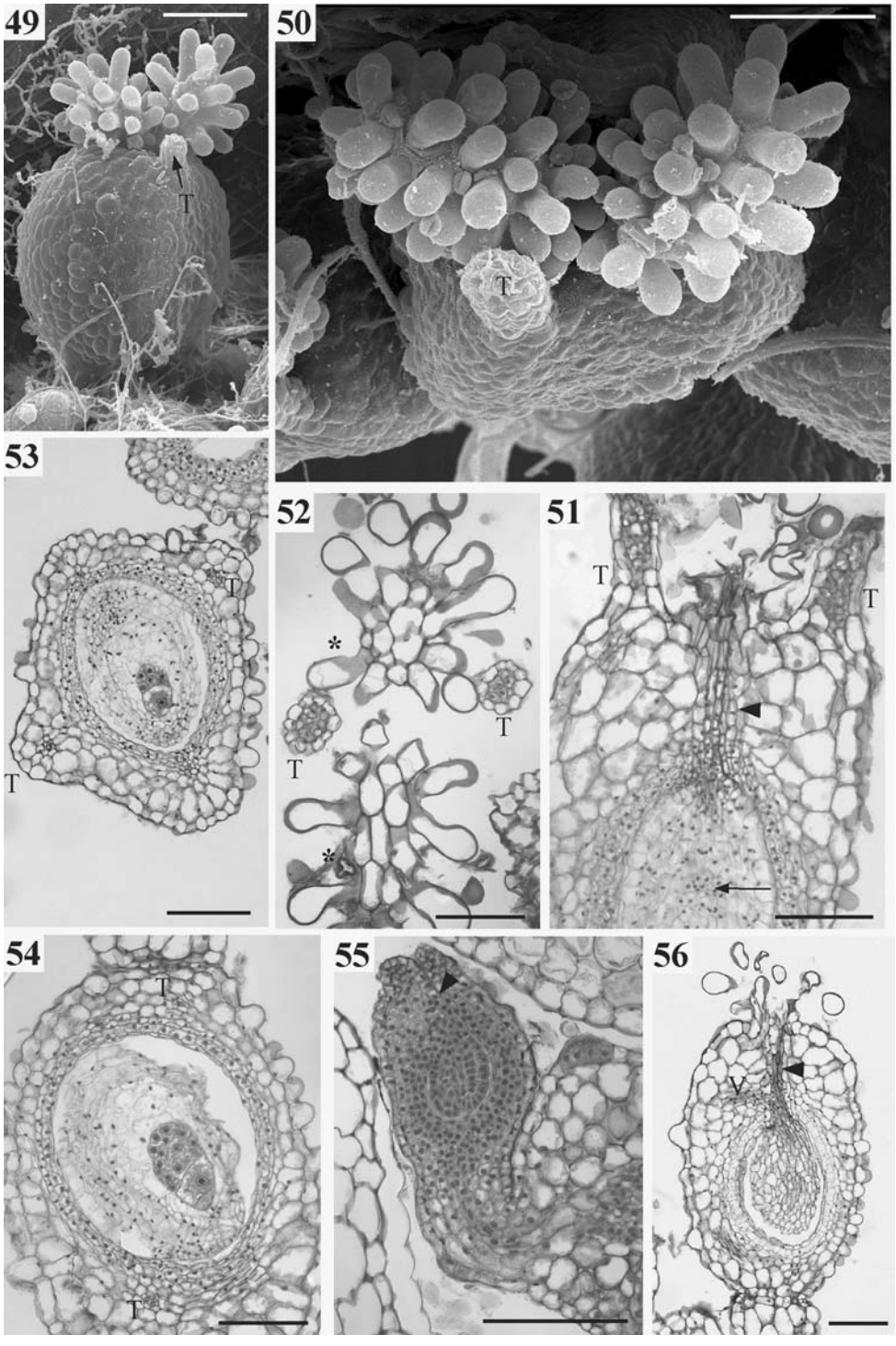
(1) *Gunnera herteri* is the only annual whereas the other *Gunnera* spp. are perennial (as already mentioned by Wanntorp et al. 2001).
(2) Mattfeld (1933) overlooked the sheath lobes of *G. herteri* foliage leaves. These sheath lobes may be interpreted as attached stipules, somewhat comparable to the stipular collar (ochrea) of *G. magellanica*.
(3) He did not discuss other possibilities to circumscribe male flowers in *G. herteri*. For example, all naked stamens at the end of an inflorescence may be accepted as parts of a single male flower.
(4) Mattfeld (1933) observed in female flowers of *G. herteri* inconspicuous stigma lobes as short as the tepal teeth. Our material, however, contained female flowers with papillate stigma lobes which are considerably larger than the tepals.
(5) He did not say anything about the ovule and young fruit, probably due to an incomplete collection.

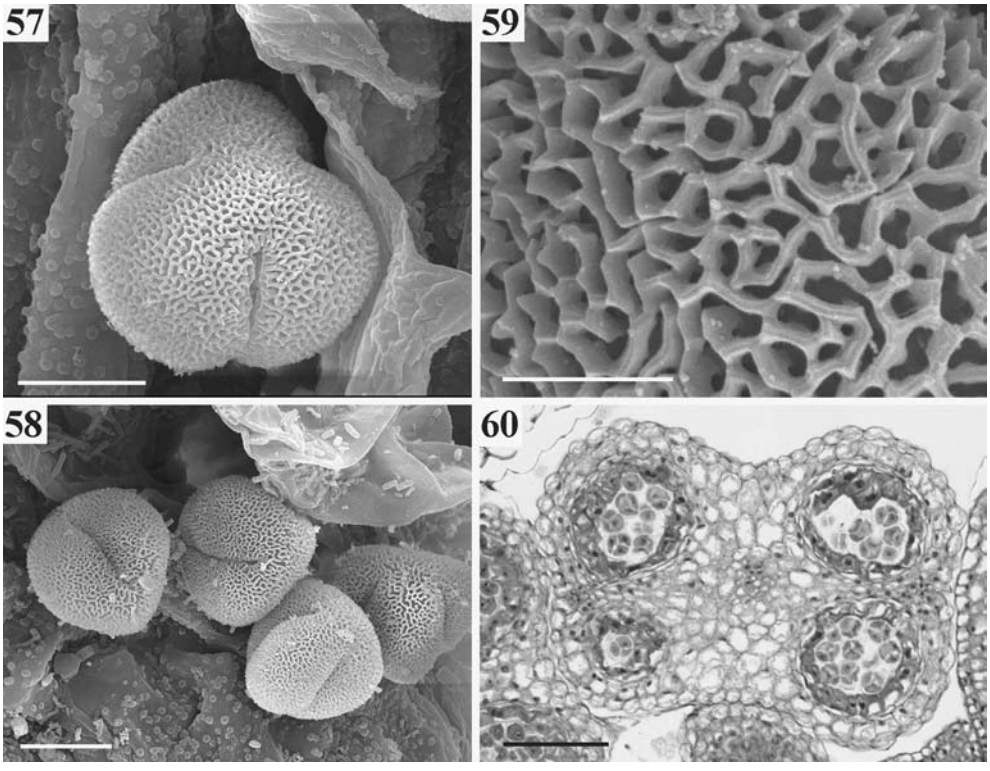
Mattfeld (1933) and Wilkinson (1998, 2000) could not study the morphology and anatomy of developing organs. Thus, some of our developmental data represent novel informations (see paragraphs I–V):

I. Sympodium hypothesis of stem growth in *Gunnera herteri*, i.e. stem interpreted as a chain of sympodial units: According to Mattfeld (1933) each inflorescence originates in the axil of a subtending leaf two or three nodes below, but are shifted upwards along the stem over two or three internodes, due to congenital fusion with the mother axis. In addition, each

Figs. 41–48. *Gunnera herteri* (cult. BG Stockholm). Inflorescence prior and during anthesis. **41, 42.** Two lateral views of same inflorescence consisting of three stamens (♂) in distal position and several female flowers (♀) further below. Some of the female flowers are arranged in triads (·–·–·). Each female flower mainly consists of an inferior ovary and two papillate stigma lobes. One female flower is marked with an asterisk in both figures. Bracts and bracteoles are lacking. Scale bars = 500 μm. **43.** Tip of inflorescence shown above, after removal of all flowers except for two female flowers (♀) and three uppermost stamens (with dehisced anthers) on common stalk (arrow). Scale bar = 400 μm. **44.** Tip of another inflorescence with four stamens consisting of undehisced anthers and filaments which are basally fused (arrow). Scale bar = 200 μm. **45.** Close-up of young inflorescence bud, showing distal male flowers (♂) and three primordial lateral branches (♀) producing female flower buds (bumps) as subunits. Scale bar = 90 μm. **46.** Close-up of distal portion of inflorescence (i.e. *I11* in Fig. 31, adjacent to leaf *L14*) with male flower buds = stamens (♂) and primordial female flowers. Only uppermost flowers are male (♂) whereas all others are female ones. Scale bar = 100 μm. **47.** Close-up of another young inflorescence with female flower buds (♀), consisting of two tepal primordia (arrowheads) and central cleft of initial inferior ovary (arrow). No bracts and bracteoles are observable. Scale bar = 60 μm. **48.** Cross-section of inflorescence showing insertion of inferior ovaries (= female flowers ♀) on short stalks which are subunits of a lateral branch (arrowhead) of main inflorescence axis (*X*). Scale bar = 400 μm

foliage leaf may subtend an additional bud which is vegetative and stays in the leaf axil. — Mattfeld did not study the initiation pattern of the inflorescence primordia as compared to the leaf primordia in growing shoot tips (see our Results). We believe that Mattfeld's "monopodium hypothesis" is wrong and should be replaced by our "sympodium hypothesis". Under this model, the upright shoot of *G. herteri* actually consists of a chain of



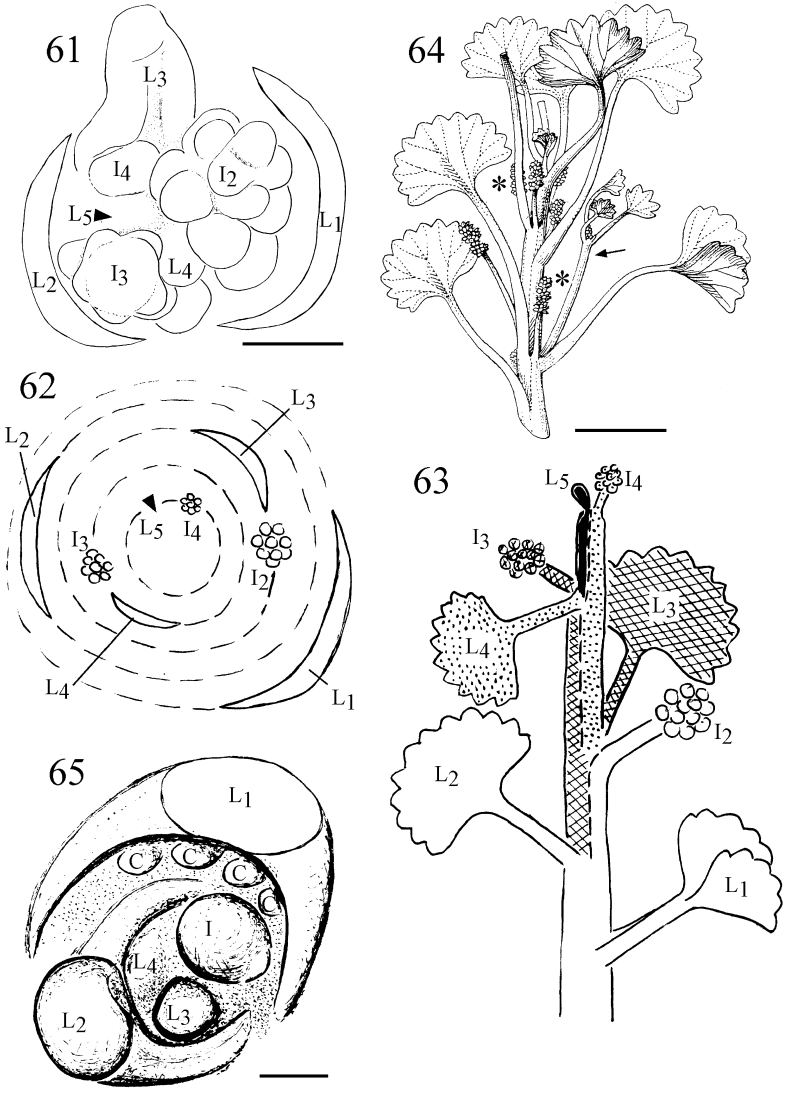


Figs. 57–60. *Gunnera herteri* (cult. BG Stockholm). Stamen and pollen structure. **57.** Tricolpate pollen grain inside pollen sac after dehiscence. Scale bar = 8 μm. **58.** Group of slightly shrunken asymmetric pollen grains inside pollen sac, only one or two colpi observable. Scale bar = 10 μm. **59.** Close-up of pollen tectum. Scale bar = 2 μm. **60.** Cross-sections of anther with four pollen sacs, containing young pollen tetrads . Scale bar = 400 μm

sympodial units, each one terminating with an inflorescence (Fig. 63). For example, the lateral shoot with two leaves and a primordial inflorescence shown in Fig. 37 can be interpreted according to the ''sympodium hypothesis'': The first shoot order terminates after the formation of two leaves with an inflorescence while in the axil of leaf 2 there is an axillary meristem forming the second sympodial unit. According to the ''sympodium hypothesis'' the two-flowered inflorescence I (shown in Fig. 40) terminates a sympodial unit with leaf 4 as its uppermost leaf and leaf 5 as the first leaf (prophyll) of the next younger shoot order. This structure is supposed to be initiated in the axil of leaf 4 but it becomes congenitally fused

Figs. 49–56. *Gunnera herteri* (cult. BG Stockholm). Anatomy of anthetic female flowers and young fruits. **49, 50.** Lateral and top view of female flower in anthesis. The inferior ovary is topped by two star-like papillate stigma lobes and two inconspicuous tepals (*T*, one hidden). Scale bars = 100 μm. **51.** Median longitudinal section of inferior ovary showing tepals (*T*), pollen-tube transmitting tissue (PTTT = arrowhead) and pendent ovule (arrow). Scale bar = 100 μm. **52.** Cross-section through stigma lobes (asterisks) and tepals (*T*) of anthetic female flower. Scale bar = 100 μm. **53, 54.** Cross-section and longitudinal section of postanthetic female flower (young fruit), showing developing embryo surrounded by abundant endosperm. Ovary wall consists of slightly papillate outer epidermis, about two large-celled parenchymatous layers and three or four inner layers of smaller cells. Note presence of vascular bundles in tepal sectors (*T*). Scale bars = 100 μm. **55, 56.** Longitudinal sections of very young and young female flower buds, showing pendent ovule primordium, vascular bundle (*V*) providing the ovule. Arrowheads point to apical channel finally serving as PTTT. Scale bars = 100 μm

over one internode with the axis of the former unit (compare for illustration Fig. 63). Finally, an elongated shoot shows ''pairs'' consisting of a foliage leaf (L) and an extra-axillary inflorescence (I) arising from the same node, but occupying different stem sectors (Fig. 64). The branching schemes (Figs. 62, 63) help to better understand the complex arrangement of leaves (L) and inflorescences (I). The L–I pairs of consecutive stem nodes are arranged along a helix. — The shoot tip shown as SEM graphs in Figs. 33, 34 is redrawn in Fig. 61 and labelled according to the ''sympodium hypothesis''. Each inflorescence Ix terminates a sympodial unit after the formation of a single foliage leaf Lx. For example, L3 is the prophyll of the bud formed in the axil of leaf L2, whereas L4 is the prophyll of the bud formed in the axil of leaf L3 (Fig. 61). What actually looks like a monopodial stem turns out to be a chain of sympodial units, each one adding a stem internode, a foliage leaf (L) and an inflorescence (I). In spite of modular stem construction the consecutive foliage leaves (i.e. L15–L18 in Figs. 33, 34; L1–L4 in Fig. 61) are arranged in a spiral approaching a Fibonacci pattern with angles of c. 137.5°. Unlike other species of *Gunnera* with modular growth (see paragraph II below), the sympodial branching in *G. herteri* is obscured by the occurrence of



axes which appear to be congenitally fused into one axis (see vertical dashed line in Fig. 63). We believe that Mattfeld (1933) was correct in postulating a congenital fusion of the stems of two branching orders. He was wrong, however, in accepting the main axis of a *G. herteri* plant as being monopodial. — Sympodial growth with units consisting of a leaf (bract), a terminal flower and an axillary meristem forming the consecutive sympodial units (as found in *G. herteri*) is also known from Solanaceae, e.g. tomato (Reinhardt and Kuhlemeier 2002). In various Solanaceae modular growth is correlated with congenital fusion of consecutive stem orders (Huber 1980).

II. Sympodial stem growth in other *Gunnera* spp.: The above-mentioned "sympodium hypothesis" is corroborated by developmental observations in perennial *Gunnera* spp. They also have a tendency towards modular shoot construction although the number of inflorescences is much smaller than the number of leaves. There is often a single inflorescence per rosette, appearing in just one season of the year. The small and stoloniferous *Gunnera* spp. (e.g. *G. magellanica*, *G. monoica*) grow as creeping herbs (Reiner 1991). After few to several leaves a stolon gives rise to one (or two) daughter stolons which arise in the axils of distal leaves. The large-leafed *Gunnera* spp. (subg. *Panke*) such as *G. manicata* have relatively short and stout stems. Wilkinson (1998, 2000) called them pachycaulous herbs. According to her (p. 240, 262) branching of the stem is not reported for most of the large-leafed species (subg. *Panke*), except e.g. *G. petaloidea* from Hawaii. Preliminary studies on *G. manicata* indicate that also in *Gunnera* subg. *Panke* stem growth is sympodial. The young inflorescence does not arise exactly in the axil of a foliage leaf (Fig. 65, redrawn from a SEM graph given by Reiner 1991). The shoot apical meristem (i.e. stem tip) itself is used up for the formation of an inflorescence (I) primordium which grows into a branched spike (up to 80 cm long in *G. manicata*) with massive peduncle. The vegetative development of the seemingly monopodial and stout stem is con-

Figs. 61–65. *Gunnera herteri* (Figs. 61 – 64) and *Gunnera manicata* (Fig. 65). Sympodial stem growth with extra-axillary inflorescences. **61.** Drawing of shoot tip shown in Fig. 33, but with labelling according to the "sympodium hypothesis" (*I* = inflorescence, *L* = leaf). The seemingly axillary inflorescence *I2* terminates a sympodial unit after having produced the leaves *L1* and *L2*. All younger primordia arise in the axil of *L2*, with *L3* as prophyll of the next sympodial unit and *I3* as its terminal inflorescence. This sympodial branching pattern is repeated in the axil of leaf *L3*, with an axillary bud producing *L4* as prophyll of the next sympodial unit and *I4* as its terminal inflorescence. The black arrowhead points to the site where we expect initiation of L5 which is prophyll of the next higher sympodial unit, arising from axil of *L4*. Scale bar = 10 μm. **62.** Diagrammatic representation of the branching pattern shown in Fig. 61. The leaves *L1–L4* are spirally inserted, approaching a Fibonacci pattern. Concentric circles (dashed lines) connect leaves and extra-axillary inflorescences which finally will appear at the same node along the mature elongated stem (compare Fig. 64). Inflorescence *I2* will be inserted at the same node as leaf *L3*; inflorescence *I3* will be inserted at the same node as leaf *L4*, and so on. **63.** Schematic lateral view of the shoot as shown in Figs. 61 and 62 (mature stage, after stem elongation). The seemingly monopodial stem consists of a chain of four sympodial units. The lowermost terminates with inflorescence *I2* after leaves *L1* and *L2*. The next two higher units (first hatched then dotted) end up after the formation a leaf (first *L3* then *L4*) and an inflorescence (first *I3* then *I4*). Note that stem internodes consist of tissue of two consecutive branch orders which are "congenitally fused". **64.** Sketch of a mature plant portion of *Gunnera herteri* (total length 4 cm, taken from Mattfeld 1933, his fig. A), showing stalked foliage leaves along elongated stem. Arrow points to vegetative axillary shoot. Extra-axillary inflorescences (asterisks) are adjacent to a leaf node each. Scale bar = 1 cm. **65.** Shoot tip of *Gunnera manicata*, seen from above (redrawn from SEM graph in Reiner 1991). Modular growth with extra-axillary inflorescence bud (*I*). Leaves *L1–L4* are arranged in a spiral: *L1–L2* (with clasp-like sheaths) belong to the first sympodial unit terminating in inflorescence I while the primordial leaves *L3–L4* start a daughter shoot in leaf axil *L2*. Note presence of primordial scales (*C*) in axil of *L1*. Scale bar = 250 μm

Table 1. Morphology and anatomy of *G. herteri* as compared to other *Gunnera* spp. (data compiled from various sources, especially Reiner 1991; Wilkinson 1998, 2000; Wanntorp and Wanntorp 2003; Wanntorp et al. 2001, 2002, 2003, and own observations; for subdivision of *Gunnera* into six subgenera see, e.g. Wanntorp et al. 2003)

	Gunnera herteri (subgenus *Ostenigunnera*)	Other *Gunnera* taxa (species or subgenera as mentioned below)
Habit	Dwarf annual herb	Mid-sized to giant perennial herbs
Stem (see more under "Inflorescence position")	Upright and frequently branching; stem internodes usually elongated, but complex due to congenital fusion of consecutive sympodial units; stolons absent	Short upright stems (forming rosettes) without prominent internodes; horizontal stolons present in subg. *Milligania* and subg. *Misandra*
Stem vascular tissue	Single stele consisting of vascular cylinder or 3–4 somewhat separate bundles	Often more complex, in *Panke* (e.g. *G. manicata*) with up to 360 separate bundles ("steles")
Sites of stem glands and *Nostoc* colonies in stem cortex	One per leaf, on left or right side of leaf insertion area	Similar in small-sized taxa; many stem glands arise along dorsal leaf insertion in *Panke*
Root anatomy and branching	Triarch; unbranched	Pentarch to polyarch; unbranched in *G. magellanica* and *G. monoica*; once-branched in *G. manicata*
Axillary (= intravaginal) scales	2–5 cylindrical colleters (i.e. tiny scales) without vascular tissue, adjacent to vegetative axillary bud	Several scales without vascular tissue in *Pseudogunnera* and *Misandra*; over hundred leaf-like scales with vascular bundles in *Panke*
Foliage leaf sheath	Narrow, with two tooth-like lobes which may be viewed as attached lateral stipules	Broader and clasp-like in *Panke*; provided with tubular sheath (ochrea) in *Misandra*
Vascular tissue in leaf petiole	Three petiole bundles, but only one leaf trace (unilacular nodes)	Similar in *Milligania* and *Misandra*; 20 – 100 bundles (traces) per petiole in *Panke*
Distribution of anomocytic stomata (leaf blade)	On both sides of blade, i.e. leaf blade amphistomatal	On both sides of blade in *G. monoica*; less / no stomata on upper side in *G. magellanica* and *G. manicata*, respectively
Inflorescence position	Extra-axillary along elongated stem, due to congenital stem fusion of consecutive sympodial units which consist of foliage leaf and terminal inflorescence each (Figs. 62–64)	Nested in rosette center, only 1–2 inflorescences but several leaves per year and unit; terminal inflorescences in *G. macrophylla* and *G. manicata* (see Fig. 65)
Sex distribution	Monoecious	Monoecious (most spp.), gynomonoecious in *G. perpensa*[ii], dioecious in *G. magellanica*
Inflorescence type and sex distribution in monoecious spp.	Double spike, with few stamens at tip and several female flowers below, arising from secondary spikes	Complex spike, often with complex distribution pattern of bisexual and unisexual flowers

Table 1 (continued)

	Gunnera herteri (subgenus *Ostenigunnera*)	Other *Gunnera* taxa (species or subgenera as mentioned below)
Bracts, bracteoles in inflorescences	Completely lacking	Often present, e.g. bracts in *G. manicata*
Flowers in monoecious spp.	Unisexual, no rudiments of opposite gender	Rarely bisexual and female (*G. perpensa*), usually unisexual, but often with rudiments of opposite gender
Perianth in female flowers	Two rudimentary tepals (which equal sepals in other *Gunnera* spp.)	Occasionally two petals in addition to two sepals ('tepals')
Male flowers	May be viewed as consisting of a single stamen each, or single terminal flower consisting of 2–7 stamens only	Regularly with two stamens per flower, rarely one stamen per flower in *G. magellanica* (Schindler 1905)
Tricolpate pollen	Exine with sinuous muri forming imperfect reticulum	Exine with perfect reticulum (Wanntorp et al. 2004)
Ovules	One per ovary	One (rarely two[iii]) per ovary
Fruits	Dry drupes, 0.6 × 0.35 mm (Osten 1932)	Dry drupes, 1–1.4 mm long in *G. monoica*; other spp. often with larger drupes (fleshy and red)

[ii]see Lowrey and Robinson (1988)
[iii]In *Gunnera chilensis* sometimes 2 ovules in bilocular ovary (Endress and Igersheim 1999, p. 353)

tinued by an axillary bud that continues with its first leaf (prophyll = L3) the Fibonacci spiral of the foliage leaves of the former mother shoot. Thus, the stout stem continues with the same spiral of foliage leaves although consecutive shoots are involved. This interpretation (i.e. organization of shoots into a seemingly monopodial stem) is corroborated by Skottsberg's (1928, his fig. 24) findings, especially a cross-section of a stem tip of *Gunnera peltata* (another *Panke* member). Young inflorescences arise from an extra-axillary position between two leaves ("zwischen zwei Blattbasen eingefügt") although the Fibonacci spiral continues in the same direction while jumping from one to the next sympodial unit. In other words, consecutive shoots are homodromous with respect to their spiral phyllotaxis, and the first leaf (prophyll) of the next shoot occupies exactly the position which is expected according to the Fibonacci pattern (with angles of c. 137.5°). Continued spiral phyllotaxes in combination with modular shoot growth are also known from other flowering plants, e.g. *Aloë* (Asphodelaceae) and *Pinguicula* (Lentibulariaceae; Rutishauser, unpublished results).

III. Axillary scales of *Gunnera herteri* as compared with those in other *Gunnera* spp. Mattfeld (1933) described the 2–5 tiny outgrowths in the foliage leaves as "scales" in spite of the fact that they are terete hair-like structures. They can be described as colleters because they secret mucilage. More prominent and more numerous axillary scales (often flattened, again without vascular tissue) are found in members of subgenus *Pseudogunnera* (*G. macrophylla*, Skottsberg 1928) and subg. *Gunnera* (*G. perpensa*). Up to more than one hundred prominent scales per leaf axil are typical for the members of subg. *Panke* (e.g. *G. manicata*). These scales have been viewed as symbiogenetic because they secret mucilage (Benson and Margulis 2002). They are often reddish and provided with few to many vascular bundles each. Occasionally such an

axillary scale can even carry a green blade (with rough surfaces), resembling the blade of the main foliage leaves (Wanntorp et al. 2003). Thus, the following evolutionary hypothesis may be proposed: What has started as hair-like glands (colleters) in the leaf axils in basal and small *Gunnera* ancestors (cf. *G. herteri*) was later transformed into prominent scales (with vascular bundles) and – rarely – even into leaf-like outgrowths with blade in the large members of subg. *Panke*! Continuum morphology and process morphology (accepting fuzzy organ identities) help to better understand such transformation series (see Rutishauser and Sattler 1986, Rutishauser and Isler 2001, Baum and Donoghue 2002, Hawkins 2002). — Overlooking the *Gunnera* transformation series from axillary colleters to leaf-like scales, there was a long-lasting debate on the morphological significance of axillary scales in *Gunnera*:

(1) Various botanists such as Berckholtz (1891), Skottsberg (1928, 1930), Mattfeld (1933: 110) and Rutishauser (1988) accepted these scales as **homologous to leaves**. Closely related to this view is the interpretation given by Wanntorp et al. (2003) who viewed the axillary scales of *Gunnera manicata* (subg. *Panke*) and *G. macrophylla* (subg. *Pseudogunnera*) as cataphylls, i.e. homologous to leaves at the base of a shoot order.

(2) Another group of botanists (e.g. Reinke 1873, Schnegg 1902, Reimnitz 1909, Schaede 1951, Benson and Margulis 2002) interpreted the axillary scales of *Gunnera manicata* (subg. *Panke*), *G. macrophylla* (subg. *Pseudogunnera*), as well as the ochrea of *Gunnera magellanica* (subg. *Misandra*) as **stipules**. Accepting the axillary scales of subg. *Panke* as supernumerary stipules, however, we have to remember that such a case is rather exceptional in angiosperms. There are only a few dicots known with an increased number of axillary stipules per leaf, e.g. *Podostemum irgangii* (Podostemaceae; Philbrick and Novelo 2001). Moreover, we have to keep in mind that *G. herteri* has (besides axillary scales) two teeth-like sheath lobes which can also be interpreted as stipules (Figs. 12, 17, 20).

(3) A third group of botanists did **avoid any decision** about the morphological significance of the axillary outgrowths in *Gunnera*. Schindler (1905) interpreted the *Panke* bud scales as ligules, i.e. a neutral structural category homologous to neither stipules nor leaves. Goebel (1933: 1637) and Troll (1939: 1289) even avoided to view the *Panke* scales as homologous to stipules, ligules or misplaced leaves. Goebel (1933) called them "axillary scales", and Troll (1939) labelled them as "intravaginal scales" resembling those of *Enhalus* and other Hydrocharitaceae.

IV. Stem glands and *Nostoc* symbiosis of *G. herteri* as compared to other *Gunnera* species. *Gunnera* is the only genus of flowering plants known for intracellular symbiosis with cyanobacteria (Fig. 16). All species of *Gunnera* live in symbiosis with *Nostoc* which invade cells of the stem cortex. According to the extensive research on the *Gunnera* – *Nostoc* symbiosis (see e.g. Uheda and Silvester 2001, Bergman and Osborne 2002, Rai et al. 2002), mucilage-producing stem glands, located adjacent to young developing leaves serve in *Gunnera* as entrance path for *Nostoc* cells. *Gunnera herteri* has disk- to funnel-shaped glands with a papillate surface, whereas other species have glands which are divided up into several papillate outgrowths, occasionally with an additional central spine as in *G. manicata* (Reiner 1991, Wanntorp et al., unpubl. data.). Young and not yet infected stem portions of *G. herteri* have channels which are lined with cytoplasma-rich cells (Figs. 13, 14). Uheda and Silvester (2001) found similar channels (serving as infection path for *Nostoc*) in *Gunnera*. Young stems of *G. herteri* show globular regions of cytoplasma-rich cortex cells which are prepared for being invaded by *Nostoc* (Fig. 15). Thus, the internal tissue nodules in the cortex where *Nostoc* will enter

the cells are defined by the host already prior to the infection. It seems that all *Gunnera* spp. behave similarly although this fact is usually not mentioned in literature or stated differently (see e.g. Bergman et al. 1992, Uheda and Silvester 2001, Bergman and Osborn 2002).

V. Comparison of *Gunnera herteri* flowers and inflorescences with those in other *Gunnera* species. *Gunnera herteri* is a dwarf, as compared to the large-leafed species (with complex inflorescences up to 80 cm long in e.g. *G. manicata*). When we accept the ancestor of all species as being somewhat larger than *G. herteri*, various characters of *G. herteri* can be seen as autapomorphies which are correlated with miniaturization (see Hanken and Wake 1993 for a general discussion on the organismal consequences of miniaturization):

(1) There are no bisexual flowers in *G. herteri*. The female flowers lack petals and stamens completely.
(2) The male flowers of *G. herteri* do not show any rudiment of the female sex, similar to *G. monoica* (subg. *Milligania*). The (usually) two stamens of each male *G. monoica* flower have a common stalk. The two scale-like tepals (sepals) are inserted on the same level as the two stamens.
(3) The male flowers of *G. herteri* are totally naked and – according to Mattfeld (1933) – reduced to a single stamen each. However, there is no proof for such a decision. We may also accept the 2–7 naked stamens at the end of an inflorescence as parts of a single male flower. In *G. manicata* terminal flowers at the end of a spike tend to be trimerous inspite of being dimerous as usual (Reiner 1991, her fig. 84).
(4) Bracts (i.e. subtending leaves) and bracteoles (i.e. prophylls) are lacking in inflorescences of *G. herteri*. Bracts and bracteoles, however, are present in many other *Gunnera* spp.
(5) Additional features of *G. herteri* as compared to other *Gunnera* spp. are summarized in Table 1 below.

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